PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Andrew Ellington

Serial No.: 09/776,252

Filed: February 2, 2001

For: SIGNALING APTAMERS THAT TRANSDUCE MOLECULAR RECOGNITION TO A DIFFERENT SIGNAL

Group Art Unit: 1634

Examiner: Forman, B. J.

Atty. Dkt. No.: CLFR:200US

CERTIFICATE OF MAILING
37 C.F.R 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-01450, on the date below:

December 21, 2005
Date

Travis M. Wohlers

DECLARATION OF ANDREW D. ELLINGTON UNDER 37 C.F.R. § 1.131

I, Andrew D. Ellington, hereby declare as follows:

1. I am the inventor of the subject matter of all claims currently pending in the referenced patent application.

2. I understand that the Patent Examiner found the claimed subject matter of the referenced application to be anticipated by U.S. Patent No. 6,680,377.

3. I am submitting this Declaration to set forth evidence that I invented the subject matter of the claimed invention prior to May 14, 1999, the priority date of U.S. Patent No. 6,680,377.

4. All of the work described in this declaration was performed in the United States.

5. Prior to May 14, 1999, I conceived of and reduced to practice a method of transducing a conformational change in a signaling aptamer upon binding a ligand to an optical signal as recited

in the pending claims. As evidence of this conception and reduction to practice I attach, as Exhibit 1, pages from laboratory notebooks kept by two members of my laboratory, Sulay Jhaveri and Romy Kirby. I have sequentially numbered the lower left-hand corner of each page in Exhibit 1 for the purpose of referencing specific pages in this declaration. The studies described in these laboratory notebook pages were conducted under my direction, and neither Sulay Jhaveri nor Romy Kirby took part in the conception of the subject matter of the pending claims. All of the studies described in the laboratory notebook pages in Exhibit 1 were completed prior to May 14, 1999, and all of these laboratory notebook pages were dated prior to May 14, 1999; however, the dates have been redacted from the copies in Exhibit 1.

6. The design, synthesis, and purification of RNA-based signaling aptamers is described at pages 2-4 of Exhibit 1. Page 3 describes the purpose and scheme for the design and use of signaling aptamers in fluorescence studies. As stated on page 3, the purpose of these studies was to create aptamers with fluorescent properties so that the binding of a ligand would induce a detectable change in fluorescence intensity. Also as stated on page 3, the study scheme involved synthesizing RNA aptamers that bind ATP, Arginine, and Theophylline, incorporating fluorescein and acridine phosphoramidites into various positions of the aptamers, and evaluating the fluorescent properties of these aptamers. In addition, the study scheme involved expanding the study to other RNA aptamers and other dyes, as well as looking at dose-dependent changes in fluorescence intensity upon binding of ligands, and testing the specificity of aptamer binding.

7. Page 2 lists the sequences of eight aptamers designed for this study. The sequences labeled ATP-R, Theo-R, and Arg-R are non-labeled aptamers known to bind ATP, Theophylline, and Arginine, respectively. The signaling aptamers were designed based on the sequences of ATP-R, Theo-R, and Arg-R. The sequences of the signaling aptamers shown on page 2 indicate the

position of either fluorescein (F) or acridine (Ac). The signaling aptamer ATP-R-Ac13 is based on the RNA aptamer ATP-R, wherein acridine is at position 13. Signaling aptamers Theo-R-Ac27 (acridine at position 27), Theo-R-Ac10 (acridine at position 10) and Theo-R-F (fluorescein at the 5' end) are signaling aptamers based on the Theo-R RNA aptamer. The signaling aptamer Arg-R-F (fluorescein at the 5' end) is based on the Arg-R RNA aptamer.

8. Page 4 indicates how certain signaling aptamers were isolated. After the chemical syntheses of aptamers ATP-R-Ac13, Theo-R-Ac27 and Theo-R-Ac10, the aptamers were run on 10% polyacrylamide gels using electrophoresis. Bands were excised that corresponded to the aptamers and the aptamers were extracted. Ultraviolet absorbency measurements were taken at 260 and 280 nm in order to determine the quantity of aptamer recovered. As shown on page 4, the following amounts of each aptamer were recovered: 3.181 nmoles of ATP-R-Ac13, 2.448 and 2.326 nmoles of Theo-R-Ac27, and 4.407 nmoles of Theo-R-Ac10.

9. I next demonstrated that the signaling aptamer, ATP-R-Ac13, selectively bound ATP. These studies are shown on pages 5-8. Looking first to page 5, it describes that 10, 50 μl aliquots of ATP, ranging in concentration from 0 to 50 mM, were prepared and added to 200 μl aliquots of ATP-R-Ac13 (0.5 μM) solution such that the final concentration of ATP in 250 μl ranged from 0 to 10 mM. Likewise, ten, 50 μl aliquots of GTP, ranging in concentration from 0 to 50 mM, were prepared and added to 200 μl aliquots of ATP-R-Ac13 (0.5 μM) solution such that the final concentration of GTP in 250 μl ranged from 0 to 10 mM. Fluorescence was measured for each sample, starting with Sample 1, which contained no ribonucleotide, and ending with Sample 10 which contained 10 mM of ribonucleotide. The graph on page 6 shows that the fluorescence intensity (as measured by Relative Fluorescence Units (RFU)) of ATP-R-Ac13 increased in the presence of increasing amounts of ATP, but not GTP, thus demonstrating a change in the

fluorescence signal of ATP-R-Ac13 upon binding ATP and the specificity of ATP-R-Ac13 for ATP. As described on pages 7 and 8, specificity studies using ATP-R-Ac13 were also performed in the presence of the ribonucleotides CTP and UTP, and GTP once again. Looking first to page 7, ATP-R-Ac13, at a concentration of 0.5 μM, was analyzed in the presence of 1000 μM of either ATP, CTP, UTP or GTP. An initial fluorescence scan was taken of the aptamer solution, then either ATP, GTP, CTP, or UTP was added and three more scans were taken of each sample with 1 minute between scans. On page 8, the results of the fluorescence scans were averaged and graphed. A fluorescence scan of water was used to establish the base line for the graph. As the graph depicts, minimal fluorescence was detected with the CTP, UTP, and GTP samples compared with a relatively strong fluorescence signal associated with the presence of ATP. This indicates the specificity of ATP-R-Ac13 for ATP over these other ribonucleotides and indicates that the increase in fluorescence signal is the result of the signaling aptamer binding its ligand.

10. I next demonstrated that the increase in fluorescence was quantitative. These studies are described on pages 9-10. Looking first to page 9, solutions of either ATP or GTP, ranging from 0 to 500 mM, were combined with 0.5 μM ATP-R-Ac13 such that the final concentration of ribonucleotide ranged from 0 to 100 mM. The fluorescence of each solution was monitored as shown on page 10. The graph and table on page 10 demonstrate the quantitative increase in fluorescence intensity emitted by ATP-R-Ac13 upon the addition of increasing amounts of ATP.

11. The design, synthesis, and purification of DNA-based, ATP-binding signaling aptamers is described at pages 11-12 of Exhibit 1. As listed on page 11, the first aptamer described has fluorescein at the 5' end and is called 28.ST3F. Fluorescein is at position 7 in the second aptamer, called 27.ST2F. The third signaling aptamer, called 28.ST1F (later termed DFL7-8), incorporates fluorescein in between positions 7 and 8.

12. As described on page 12, the aptamers were purified by polyacrylamide gel electrophoresis and precipitated. Page 12 also depicts a binding buffer composition (NaCl, Tris, $MgCl_2$ and water) that was prepared and tested.

13. I next determined that there was a change in fluorescence behavior of DFL7-8 upon the addition of ATP. As shown on page 13, Relative Fluorescence Units (RFU, middle column) increased as increasing concentrations of ATP (right-hand column) were added to the DFL7-8 solution (49 nM DFL7-8).

14. I next determined that the increase in fluorescence intensity was quantitative. As described on page 14, one milliliter of the aptamer solution (150 nM DFL7-8) was pipetted into a fluorimeter cell, and 20 mM ATP was added in 2 μL aliquots such that the concentration of ATP was gradually increased from 0 to 600 μM. As recorded on page 14, the relative fluorescence intensity increased with the corresponding increase in ATP concentration, thus demonstrating that fluorescence detection of ATP using the DFL7-8 aptamer is quantitative. The specificity of DFL7-8 for ATP and not GTP was also demonstrated. Two microliter increments of 20 mM solutions of GTP were added to a solution of DFL7-8 (150 nM) such that the concentration of GTP was gradually increased from 0 to 600 μM and the fluorescence measured. As the graph on page 14 indicates, no fluorescence response was detected upon the addition of increasing amounts of GTP. Thus, DFL7-8 is specific for ATP.

15. Other ribonucleotides proved unresponsive in fluorescence studies with DFL7-8, as demonstrated on page 15, thus bolstering the evidence that the signaling aptamer is specific for ATP. To a 150 nM solution of DFL7-8 were added increasing concentrations (200, 400 and 600 μM) of either GTP, CTP or UTP, and fluorescence measurements were taken. The graph and table

on page 15 indicate that GTP, CTP and UTP showed no fluorescence response in comparison to the strong response seen with ATP. Thus, DFL7-8 is specific for ATP over the other ribonucleotides.

16. I next demonstrated that DFL7-8 could be used to detect ATP in a smaller volume on a plate. As shown on page 16, an increase in fluorescence intensity resulted when ATP was added in increasing concentrations to a smaller volume (150 μL) of DFL7-8 (100 nM) on a plate. Fluorescence measurements were taken using a fluorescence plate reader. As shown in the table on page 16, as the concentration of ATP increased (left-hand column), the relative fluorescence intensity increased (right-hand column).

17. To demonstrate that the signaling capability of DFL7-8 was due to the presence of the fluorescein molecule and to the signaling aptamer's binding of its target, two additional aptamers were constructed as controls and used in ATP binding studies. As described on page 17, these two additional aptamers were a nonfluorescently labeled aptamer that binds ATP (NF-FL), and a DFL7-8 mutant incapable of binding ATP (Mut DFL7-8). ATP (250 μM) was added to solutions of DFL7-8, NF-FL, and Mut DFL 7-8 and the fluorescence measured. As the graph on page 17 indicates, the NF-FL and mutant aptamers showed no fluorescence response relative to that seen with DFL7-8. This demonstrated that the conformational changes in DFL7-8 upon binding of ATP are responsible for the change in fluorescence intensity.

18. In conclusion, I reduced to practice a method of transducing a conformational change in a signaling aptamer upon binding a ligand to an optical signal as recited in the current claims prior to May 14, 1999. As described above and in Exhibit 1, I designed and evaluated both RNA-based and DNA-based signaling aptamers labeled with either fluorescein or acridine reporter molecules. Furthermore, I demonstrated that upon binding to their ligand, these signaling aptamers underwent a conformational change that produced a detectable increase in fluorescense intensity. I also

demonstrated that these signaling aptamers specifically bound their ligand, and that the increase in fluorescence intensity in the presence of ligand was quantitative.

19. I hereby declare that all statements made of my own knowledge are true and all statements made on information are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date: 12/21/05

Andrew D. Ellington, Ph.D.

Kirby, Romy

Ellington #1

LAB BOOK

SmAptamers

Romy Kirby

60 SHEETS • 5 x 5 QUAD
$10^1/_8$ x $7^7/_8$ • 53-108
National Brand
Made in USA

0 73333 53108 4

AVERY DENNISON OFFICE PRODUCTS, CH[illegible] MA 01022

Signaling Aptamers (Ac = acridine; F = fluorescein)

ATP-R 5' ggg UUg ggA AgA AAC UgU ggC ACU UCg gUg CCA gCA ACC C 3'

ATP-R-Ac13 5' ggg UUg ggA AgA (Ac)AC UgU ggC ACU UCg gUg CCA gCA ACC C 3'

Theo-R 5' ggC gAU ACC AgC CgA AAg gCC CUU ggC AgC gUC 3'

Theo-R-Ac27 5' ggC gAU ACC AgC CgA AAg gCC CUU gg(Ac) AgC gUC 3'

Theo-R-Ac10 5' ggC gAU ACC (Ac)gC CgA AAg gCC CUU ggC AgC gUC 3'

Theo-R-F 5' Fgg CgA UAC CAg CCg AAA ggC CCU Ugg CAg CgU C 3'

Arg-R 5' gAC Agg UAg gUC gCA CgA AAg UgA Agg AgC gUC 3'

Arg-R-F 5' gAC Agg UAg gUC gCA CgA AAg UgA Agg AgC gUC 3'

Signaling Aptamers

Purpose: create aptamers w/ fluorescent properties so that binding of a ligand induces a detectable change in fluorescence intensity.

Scheme: synthesize RNA aptamers which bind ATP, Arg & Theo. Explore fluorescent properties with the incorporation of fluorescein & Acridine phosphoramidites in ~~various~~ various positions of the aptamers. Expand to incorporate other RNA aptamers, and possible other dyes. Look at dose-dependent changes in fluorescence intensity upon binding of ligands. Test for specificity in binding (ATP & GTP, Arg & Cit, Theo & caffeine)

Synthesized aptamers

ATP-R, ATP-R-Ac13

Theo-R-Ac27, Theo-R-Ac10

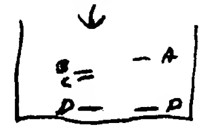
1.5 thick 10 tooth comb 10% 250V 2hrs 40μl sample 6 lanes

neither aptamer was fluorescent yellow after purification

isolate 2nd ATP-R-Ac13 & 1st Theo-R-Ac27 from gels

gel purify 2nd Theo-R-Ac27 & 1st Theo-R-Ac10

two bands (B & C) for theo-R-Ac10 →



A 40mer
D Dye

1.5 thick 10 tooth 10% 250V 2hrs
40μl sample 6 lanes

gel purify 2nd Theo-R-Ac 10

isolate 2nd Theo-R-Ac27
1st Theo-R-Ac10 upper band & lower band

1.5 thick 10 tooth
10% 250 V 2hrs
40μl sample 6 lanes

isolate 2nd Theo-R-Ac10 upper band

Quantitation - Pure

	260	280 (~~260~~)	260/280	μM c	nMoles	Sample V	Suspension V
2nd ATP-R-Ac13	0.021	0.011	1.901	63.63	3.181	1μL	50 μl
1st Theo-R-Ac27	0.020	0.011	1.772	48.97	2.448	1.5μl	50 μl
2nd Theo-R-Ac27	0.019	0.011	1.717	46.52	2.326	1.5μl	50 μl
1st Theo-R-Ac10 upper	0.024	0.014	1.754	88.15	4.407	1 μl	50 μl

all H_2O

1) stock soln of aptamer (0.5µM aptamer/ 1x PBS)

3mL 158.4µL H_2O
800 µL 5x PBS buffer
41.6µL ATP-R-Ac13 (51.51µM) 1st pure

total 4mL stock soln 0.5µM aptamer / 1x PBS

Divide into 20 200µL aliquots

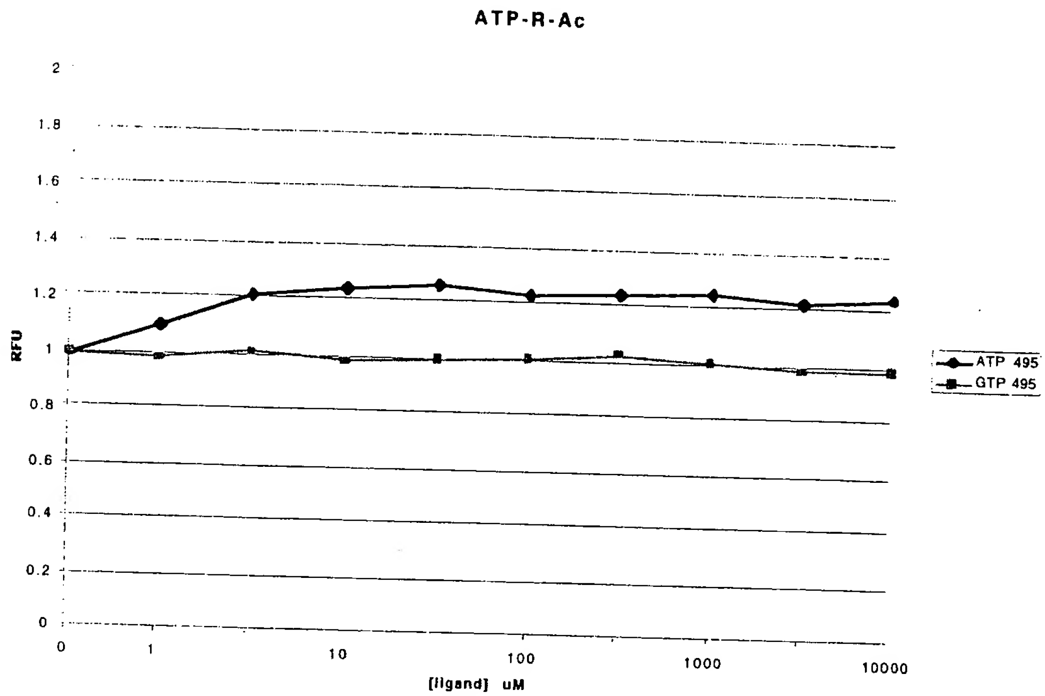
2) Dilutions

Sample	Ligand (100mM)	H_2O	[ligand]	50µL soln [ligand] in 250µL	* Binding Aptamer Soln RFU
10	50 µL	50µL	50mM	10 mM	
9	31.6µL of 10	68.~~38~~4µL	15.81 mM	3.162 mM	
8	of 9	68.4	5mM	1 mM	
7	of 8		1581 µM	316.2 µM	
6	of 7		500 µM	100 µM	
5	of 6		158.1 µM	31.62 µM	
4	of 5		50 µM	10 µM	
3	of 4		15.81 µM	3.162 µM	
2	of 3		5µM	1 µM	
1	0	50 µL	0	0	

3) make up samples for GTP/Aptamer solution & ATP/Aptamer solution

11 20 aliquots → 200µL stock aptamer solution
50µL ligand solution
250µL Binding Solution

4) measure fluorescence of each sample → starting with sample 1 (0 ligand) & proceeding up to sample 10 (10mM ligan



RKA01.DAT - RKA10.DAT **RKG01.DAT - RKG10.DAT**

Sample	A495	A530	G495	G530	conc-uM
1	3.11447	3.28201	3.56201	3.99216	0
2	3.42407	3.47046	3.49945	3.82736	1
3	3.77411	3.69278	3.58887	3.9502	3.162
4	3.8591	3.73932	3.48633	3.90625	10
5	3.93265	3.91541	3.53668	3.92242	31.62
6	3.83698	3.71353	3.582	3.97202	100
7	3.8649	3.69736	3.64883	4.03	316.2
8	3.89938	3.79211	3.56522	3.99689	1000
9	3.80981	3.55057	3.49365	3.86948	3162
10	3.86932	3.38364	3.50449	3.78021	10000

Check heat denaturing conditions for
ATP-R-Ac Batch 3 2nd ½ 1st pure

heat denature in heat block 65°C for 3 min & remove — allow
to cool to room temp ≈15 min

ATP			[ligand]
100 mM × 1 μL		501 →	199.6 μM
"	"	502 →	398.8
"	"	503 →	597.6
"	"	504 →	796.0
"	"	505 →	994.0

H_2O	397.96 μL
5x tris	100 μL
Aptamer	2.04 μL
	500 μL

Check ATP-R-Ac Batch 3 1st ½ 2nd pure
against ATP, GTP, UTP, CTP @ 1000 μM [ligand]

aptamer @ 0.5 μM

have 5 samples each containing 250 μL of 0.5 μM aptamer

scan aptamer soln ~~4 times~~ & then add
2.5 μL of 100 mM ATP & scan 3 more times
" " GTP
" " CTP
" " UTP
H_2O — standard

4 times

scans 2-4 were lower than the 1st scan

wait 1 min b/t scans

print out bar graph of RFU increase - using the H_2O Standard as the baseline

µM [ligand]	ATP	Avg	Stdev	Inc RFU A	GTP	Avg	Stdev	Inc RFU G
0	3.56644	3.549707	0.014516	0	3.73352	3.701020	0.030708	0
	3.54218				3.67249			
	3.5405				3.69705			
1000	4.13834	4.121603	0.014600	0.16111	3.5524	3.519743	0.028726	-0.04898
	4.11499				3.49838			
	4.11148				3.50845			

µM [ligand]	CTP	Avg	Stdev	Inc RFU C	UTP	Avg	Stdev	Inc RFU U
0	3.45215	3.444623	0.010991	0	3.43536	3.425953	0.011425	0
	3.43201				3.41324			
	3.44971				3.42926			
1000	3.20541	3.197987	0.006436	-0.07160	3.32565	3.290457	0.030484	-0.03955
	3.19397				3.27347			
	3.19458				3.27225			

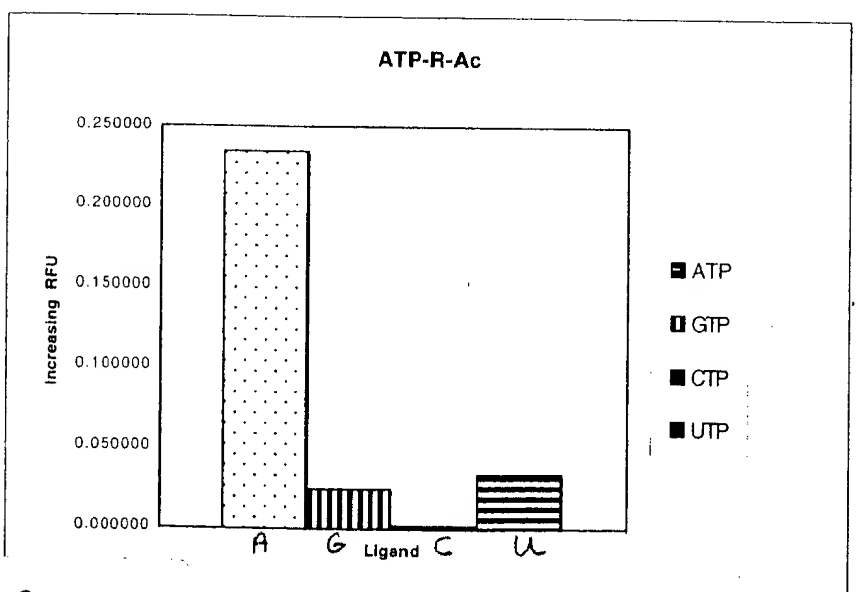
ATP
100m
"
"
"
"

BASELINE check decrease in RFU from addition of 2.5mL volume

µL H20	H2O	Avg	Stdev	Inc RFU H
0	2.93228	2.935843	0.012589	0
	2.94983			
	2.92542			
2.5	2.72705	2.720947	0.006181	-0.07320
	2.71469			
	2.7211			

Incr RFU

Inc RFU A	Inc RFU G	Inc RFU C	Inc RFU U
0.234309	0.024217	0.001597	0.033648



check change in fluorescence upon addition of ATP & GTP to ATP-R-Ac Batch3 2nd

ATP & GTP Dilutions

Stock ATP & GTP soln 500mM in Tris buffer (20 mM tris pH 7.6, 300mM NaCl, 5mM $MgCl_2$)

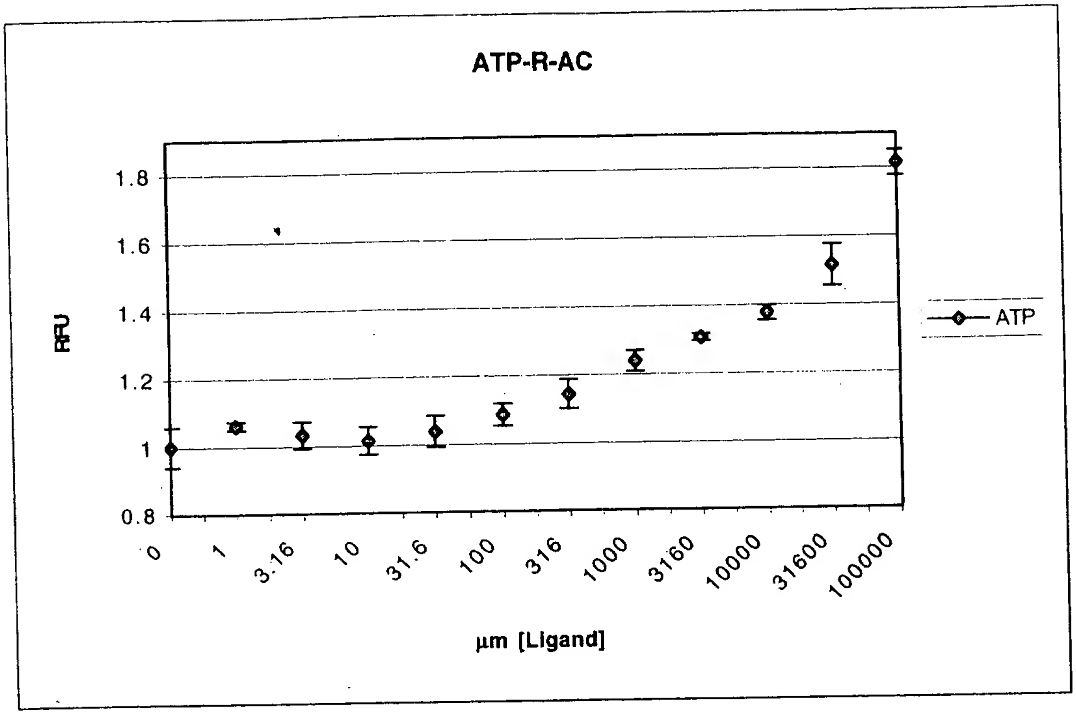
Soln	Ligand		Buffer	[ligand]		[ligand] in 250µL
12	100µL 500mM ligand	+	0µL →	500mM	→	100 mM
11	31.6µL of 12	+	68.4µL →	158 mM	→	31.6mM
10	31.6µL of 11		→	50 mM	→	10 mM
9	of 10		→	15.8 mM	→	3.16 mM
8	of 9		→	5 mM	→	1 mM
7	of 8		→	1.58mM	→	316 µM
6	of 7		→	500 µM	→	100 µM
5	of 6		→	158 µM	→	31.6 µM
4	of 5		→	50 µM	→	10 µM
3	of 4		→	15.8 µM	→	3.16 µM
2	of 3		→	5 µM	→	1 µM
1	0µL	+	100µL →	0µM	→	0 µM

ATP-R-Ac soln

3830.68 µL H_2O
960 µL 5x Tris buffer
9.32 µL ATP-R-Ac batch 3 2nd
4800µL

0.5µM in 6000µL total :
⇓
24x 200µL aliquots w
24x 50µL aliquots
aptamer ↑ ligand

divide into 24 200µL aliquots



Sample	µM [ligand]	ATP	Avg	Stdev	RFU ATP
1	0	3.61038	3.65212	0.059022	1
		3.69385			
2	1	3.86948	3.87833	0.012516	1.061941
		3.88718			
3	3.16	3.79791	3.76930	0.040461	1.032087
		3.74069			
4	10	3.73215	3.70354	0.040461	1.014081
		3.67493			
5	31.6	3.76053	3.79364	0.046825	1.038752
		3.82675			
6	100	3.99124	3.96828	0.032477	1.086569
		3.94531			
7	316	4.20822	4.17793	0.042837	1.143975
		4.14764			
8	1000	4.54559	4.52377	0.030858	1.238671
		4.50195			
9	3160	4.77753	4.76990	0.010790	1.306065
		4.76227			
10	10000	5.04364	5.02793	0.022224	1.376716
		5.01221			
11	31600	5.57358	5.53048	0.060960	1.514321
		5.48737			
12	100000	6.64993	6.62346	0.037441	1.813594
		6.59698			

Seuqunces of Fluorosceinated DNA ATP aptamers

1) 5' -FLU-ACC,TGG,GGG,AGT,ATT,GCG,GAG,GAA,GGT
2) 5'- ACC,TGG,-FLU-GG,AGT,ATT,GCG,GAG,GAA,GGT
3) 5'- ACC,TGG,G-FLU-G,GAG,TAT,TGC,GGA,GGA,AGG,T

1 28. SJ3F

2 27. SJ2F

3 28. SJ1F

precipitate fluorescent oligo's gel purified.

Fl# 1	5' F	DNA aptamer	28 mer
Fl# 2	#7 F	instead of G	27 mer
Fl# 3	7-F-8		28 mer.

Make up binding buffer a la Huizenga et al.

		10x	Stocks	for 50ml 10x
300 mM	NaCl	3M	5M	30
20 mM	Tris pH 7.5	200mM	1M	10
5 mM	MgCl2	50mM	1M	2.5
				+ 7.5 ml H_2O

Test w/ #2
see SJ023098.dat1, dat2

2 ul SJ1F in 1 ml ATP FBB. [ATP]

Remove 1/2, 1/4, 1/8. so [SJ1F] = 49 nM

	RFU	[ATP]
	RFU = 6.06	0
add 1 ul 17 nM ATP.	RFU: 5.99	17 p
add 1 ul 81 nM A.	RFU: 6.05	98 p
" 386 nM A	6.06	484
1.8 uM A	6.15	2284
8.7 u	6.72	10980
41 u	6.86	5.2 x 10^4
196 u	6.85	2.48 x 10^5 ~~247984~~
4.4 m	7.13	4.65 x 10^6 ~~4647784~~
21 m	7.33	2.56 x 10^7
100 m	7.69	1.26 x 10^8
100 m	7.96	2.26 x 10^8
4 ul 100 mM	8.04	6.26 x 10^8

1.9 10x PNK Bf
.9 PNK
5 ATP γ^{32} YICN
1 H_2O

...ter

SMART stuff:
SJ3 7-F-8
150 nM.

~~ATP~~ ~~RFU~~

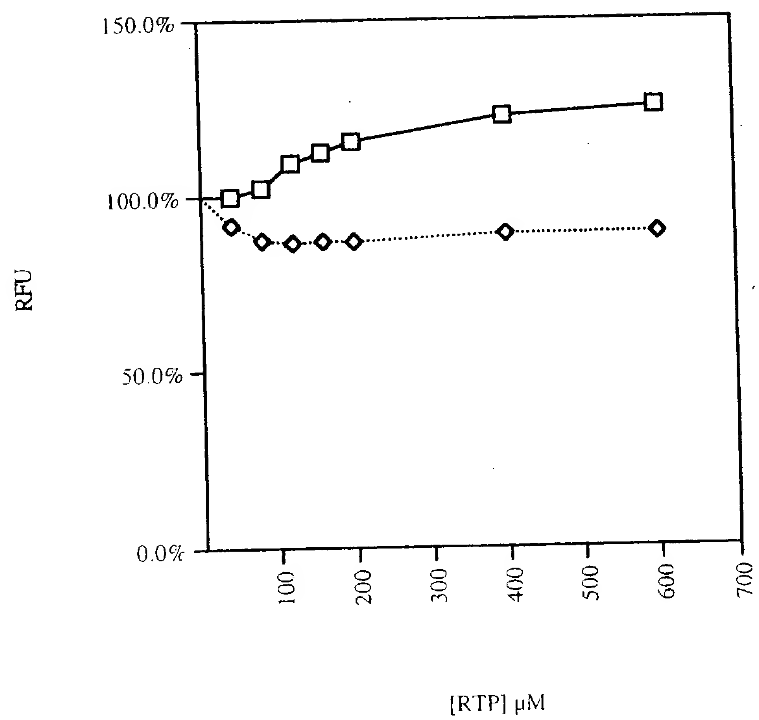
~~0~~	~~4.483~~
~~40 μM~~	~~4.589~~
~~80~~	~~4.902~~
~~120~~	~~5.039~~
~~160~~	~~5.182~~
~~200 μM~~	~~5.495~~
	~~5.615~~

ATP	
0	4.488
40	4.483
80	4.585
120	4.902
160	5.039
200	5.182
400	5.495
600	5.615

ATP, GTP @ 20 mM
add 2 ul at a time

GTP	RFU.
0	4.499
40 μM	4.589
80	4.368
120	4.3272
160	4.3485
200 μM	4.3466
400	4.4486
600	4.46

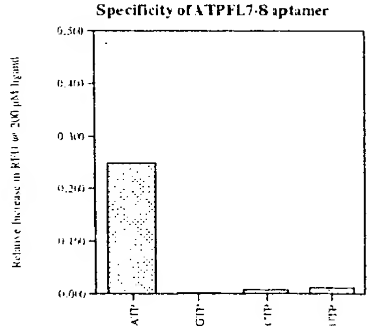
SJ3 A &G



Data #1

	1	2	3	4	5
	RFU ATP	RFU GTP	RFU CTP	RFU UTP	[NTP] µM
1	5.2191	4.46564	4.61243	4.442	0
2	6.52779	4.472	4.64291	4.492	200
3	6.874	4.550	4.646	4.508	400
4	7.054	4.582	4.702	4.568	600
5					
6					

Specificity of ATPFL7-8 aptamer



Protocol for FL7-8 studies.

Add 996 µl 150 nM ATPFL7-8 to cuvette

Obtain Sensitivity ~ 600-700 eV. 50%.

Emission scan exc @ 494-497 4 nm bandpass.

emm @ 514-518

add 2 µl aptamer soln scan again

repeat.

scan 3 more times until RFU signal stabilizes

add 2 µl 100 mM NTP.

Assessing complexity of N40 pool/PCR.

5' label 18.40.

1ul primer ~~P.P.~~ 171 pmol.
1ul ATP P^{32} ICN 8
3ul 10x PNK buffer
1ul T4 PNK
2.3ul H_2O

phenol chloroform / chloroform extract
precipitate.
~~add~~ resuspend in 10ul H_2O
add 1ul to 2ul pool. + PCR mix.
95° 1 min
45° 1 min
20 min ext.

Fun w/ the new plate reader

100nM DFL7-8
150ul volume

1500	28177	612	0.02172	0.420426	0.009132	1500
750	26410	1050	0.039758	0.331351	0.013174	750
375	25763	1360	0.052789	0.298735	0.01577	375
187.5	24479	1540	0.062911	0.234007	0.014722	187.5
93.75	22723	1358	0.059763	0.145486	0.008695	93.75
46.875	21399	620	0.028973	0.078742	0.002281	46.875
23.438	21302	669	0.031406	0.073852	0.002319	23.438
11.719	21144	669	0.03164	0.065887	0.002085	11.719
5.8594	20814	494	0.023734	0.049251	0.001169	5.8594
2.9297	20932	1089	0.052026	0.0552	0.002872	2.9297
[ATP]	Mean RFU	STD RFU		%inc RFU		

add 40 ~ 500mM ATP

KC4

Protocol Description

Name :	NONAME.PRT		**MODIFIED**
Reader :	FL600	Wells :	B3-D8
Reading Direction :	HORIZONTAL	Lag Time :	00:00:00
Shaking :	No	Plate Type :	Costar 96 well half area
Wavelength :	485/20 / 530/25 nm	Sensitivity :	100
Optics position :	Top		

Assay Description

Data Name:	NONAME.PLA		
Reading Type:	Reader		
Reading Date:		Report Date:	
Prompt #1:		Prompt #4:	
Prompt #2:		Prompt #5:	
Prompt #3:		Prompt #6:	
Comments:			

STATISTICS - M 485/530

Identif.	Name	Conc\Dil	Well	485/530	Nb	Mean	Std Dev	CV (%)
SPL1		1.0000	B3	35954	3	35740	1913.5	5.3538
			B4	37538				
			B5	33729				
SPL2		1.0000	B6	39424	3	37400	1768.4	4.7284
			B7	36625				
			B8	36152				
SPL3		1.0000	C3	55144	3	54430	620.36	1.1397
			C4	54127				
			C5	54020				
SPL4		1.0000	C6	54152	3	53925	596.43	1.1060
			C7	54374				
			C8	53248				
SPL5		1.0000	D3	17444	3	17502	136.21	0.7783
			D4	17405				
			D5	17658				
SPL6		1.0000	D6	17600	3	17567	182.21	1.0372
			D7	17371				
			D8	17731				

35740	37799	2059	0.057611	0.296721	DFL7-8
37400	50652	13252	0.354332		
54430	59048	4618	0.084843	-0.011797	NF + FL
53925	57864	3939	0.073046		
17502	17858	356	0.020341	-0.03275	Mut DFL7-8
17567	17349	-218	-0.01241		

250 micro molar ATP